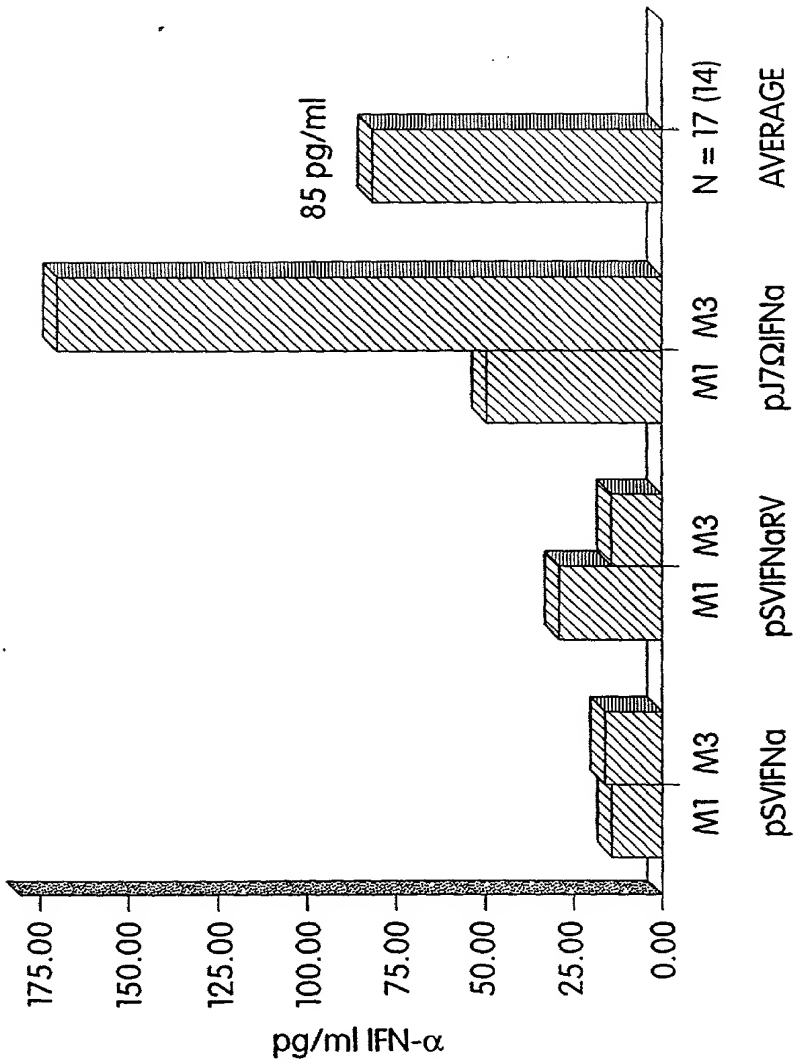
175.00
150.00
125.00
100.00
75.00
50.00
25.00
0.00
pg/ml IFN-α
85 pg/ml
M1 M3
pSVIFNa
M1 M3
pSVIFNaRV
M1 M3
pJ7ΩIFNa
N = 17 (14)
AVERAGE

Fig. 1

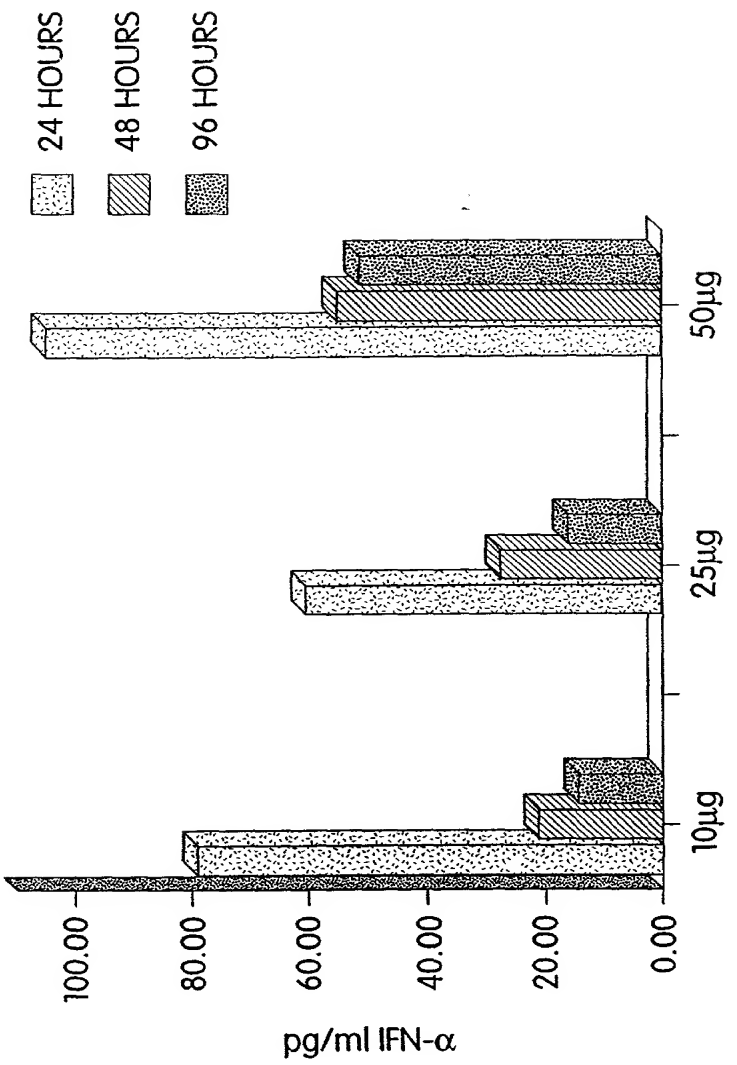
24 HOURS
48 HOURS
96 HOURS
100.00
80.00
60.00
40.00
20.00
0.00
pg/ml IFN-α
10µg
25µg
50µg
DOSE INJECTED

Fig. 2

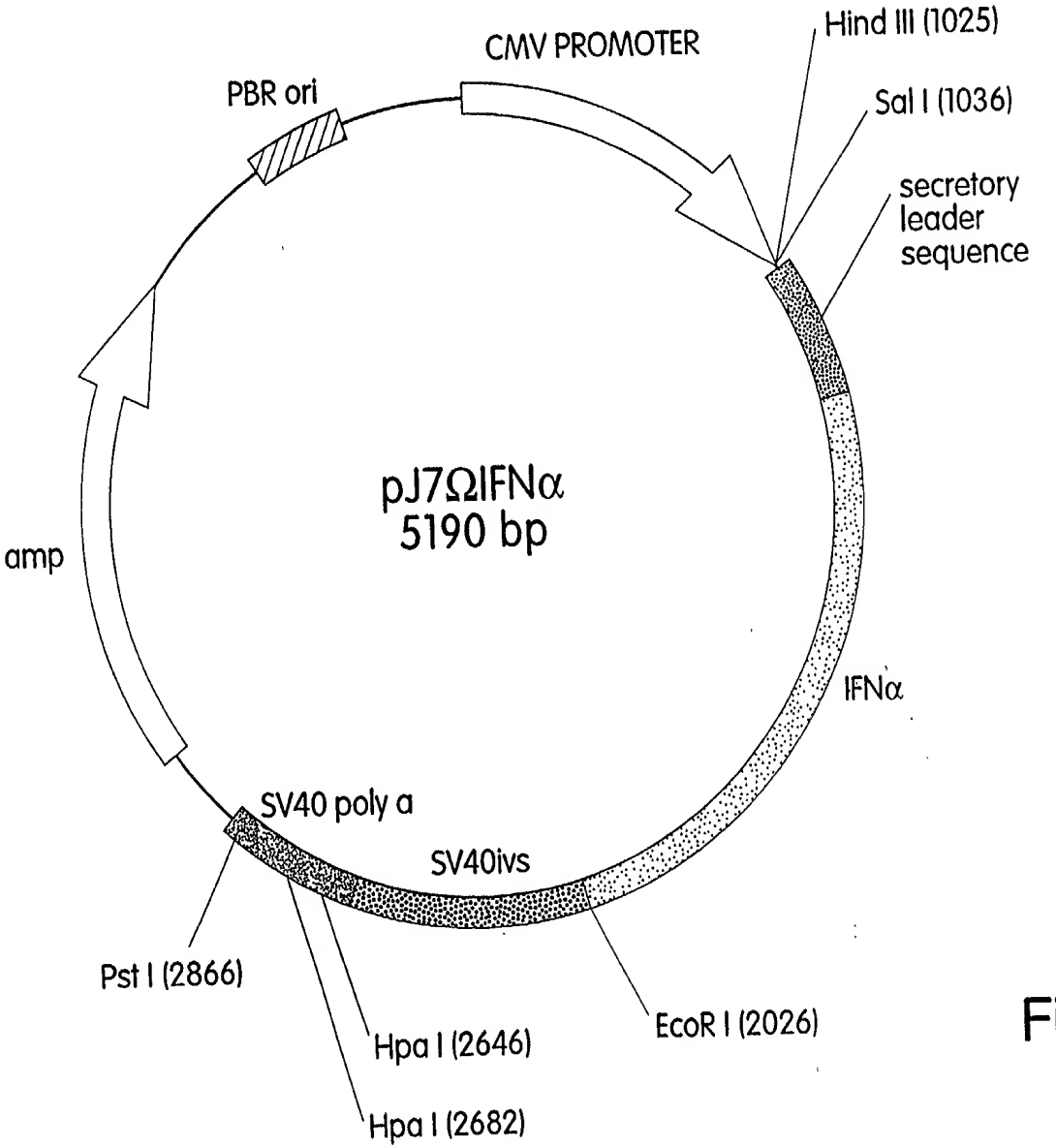
CMV PROMOTER
Hind III (1025)
Sal I (1036)
secretory
leader
sequence
PBR ori
pJ7ΩIFNα
5190 bp
amp
IFNα
SV40 poly a
SV40ivs
Pst I (2866)
Hpa I (2646)
Hpa I (2682)
EcoR I (2026)

Fig. 3

FOGARTY 52hh6560

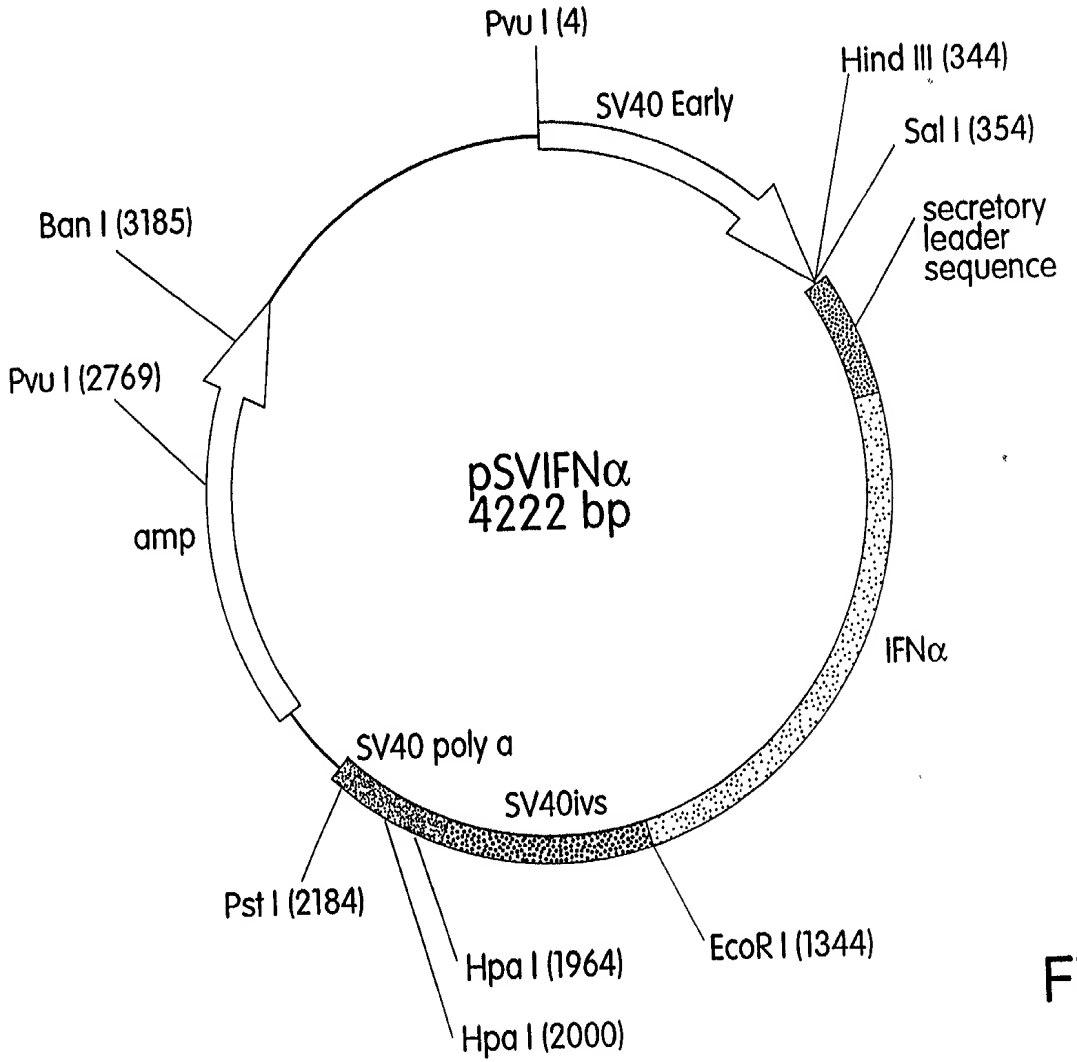

Fig. 4

TO92TT' S2hmb660

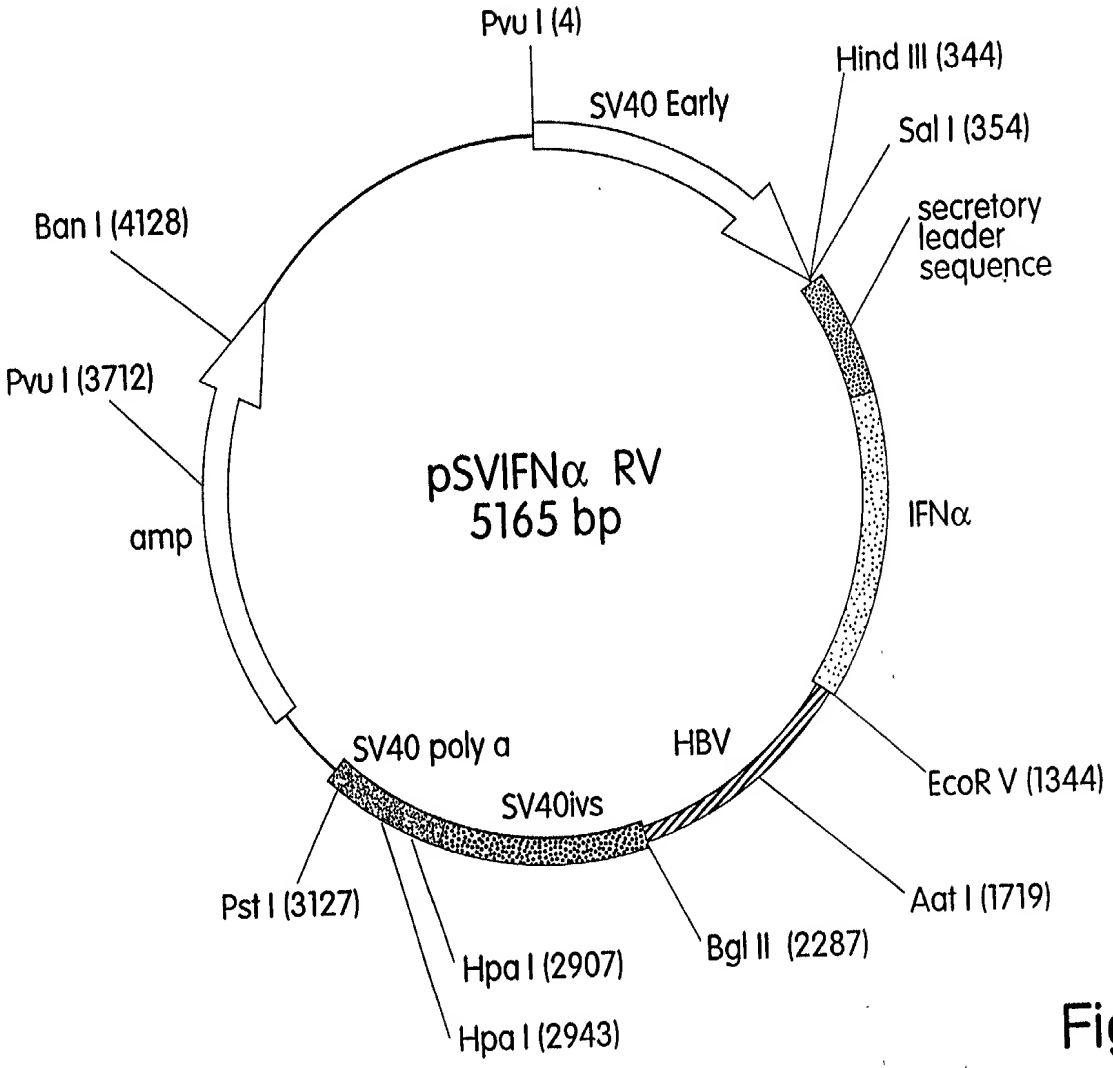

Fig. 5

TO92TT S2HH6660

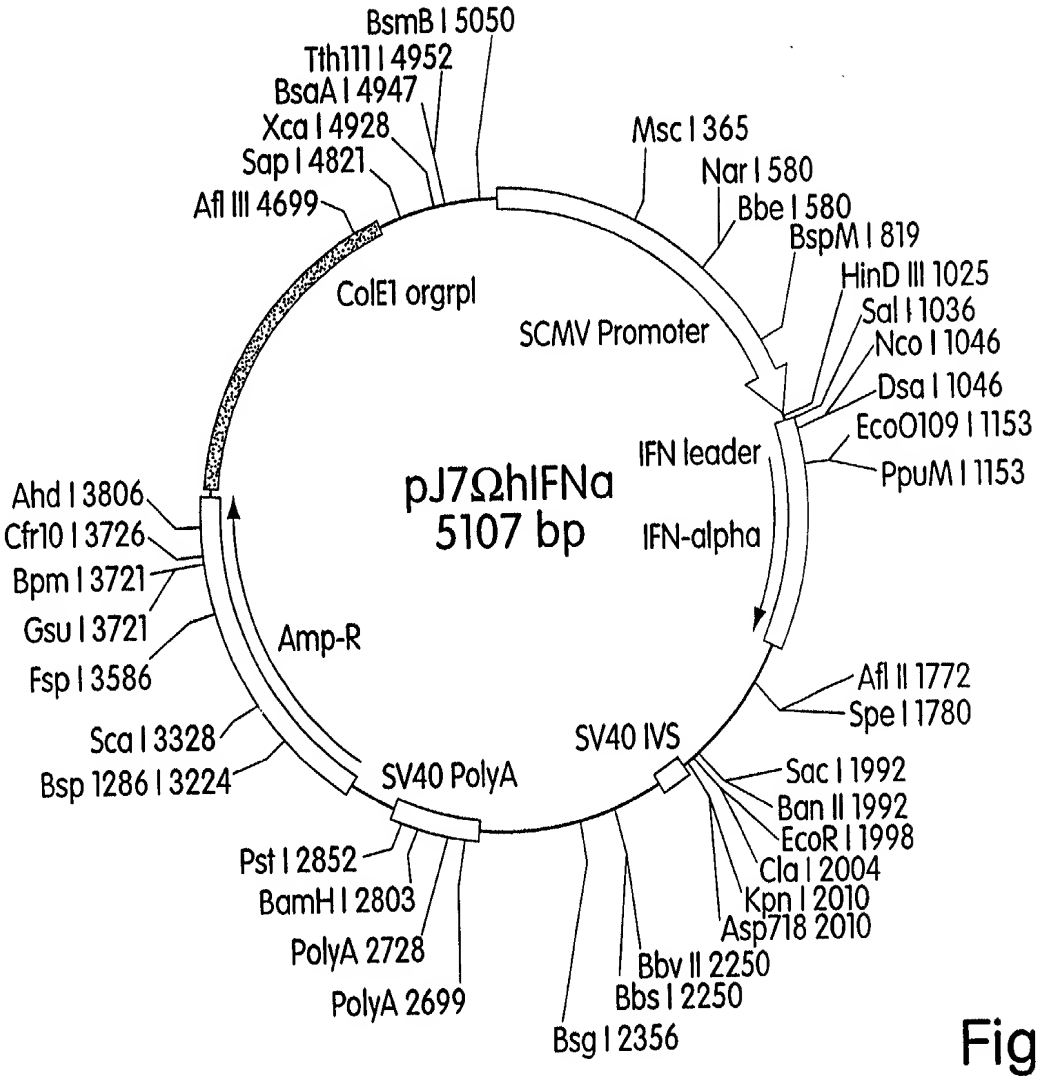

Fig. 6

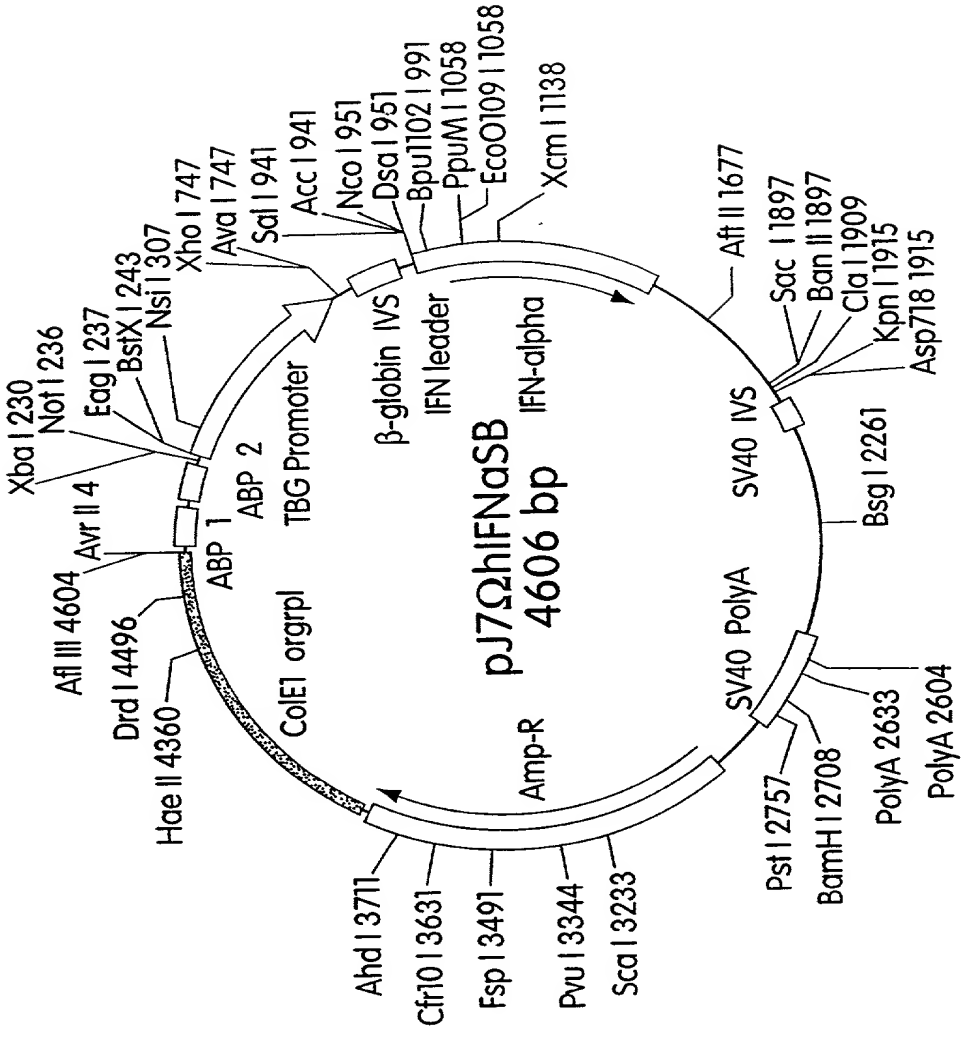
pJ7ΩhIFNαSB
4606 bp
Xba I 230
Not I 236
Eag I 237
BstX I 243
Nsi I 307
Xho I 747
Ava I 747
Sal I 941
Acc I 941
Nco I 951
Dsa I 951
Bpu1102 I 991
PpuM I 1058
Eco0109 I 1058
Xcm I 1138
Aft II 1677
Sac I 1897
Ban II 1897
Cla I 1909
Kpn I 1915
Asp718 1915
Bsg I 2261
PolyA 2604
PolyA 2633
BamH I 2708
Pst I 2757
Sca I 3233
Pvu I 3344
Fsp I 3491
Cfr10 I 3631
Ahd I 3711
Hae II 4360
Drd I 4496
Afl III 4604
Avr II 4
ABP 1
ABP 2
TBG Promoter
ColE1 orgrpl
Amp-R
SV40 PolyA
SV40 IVS
IFN-alpha
IFN leader
β-globin IVS

Fig. 7

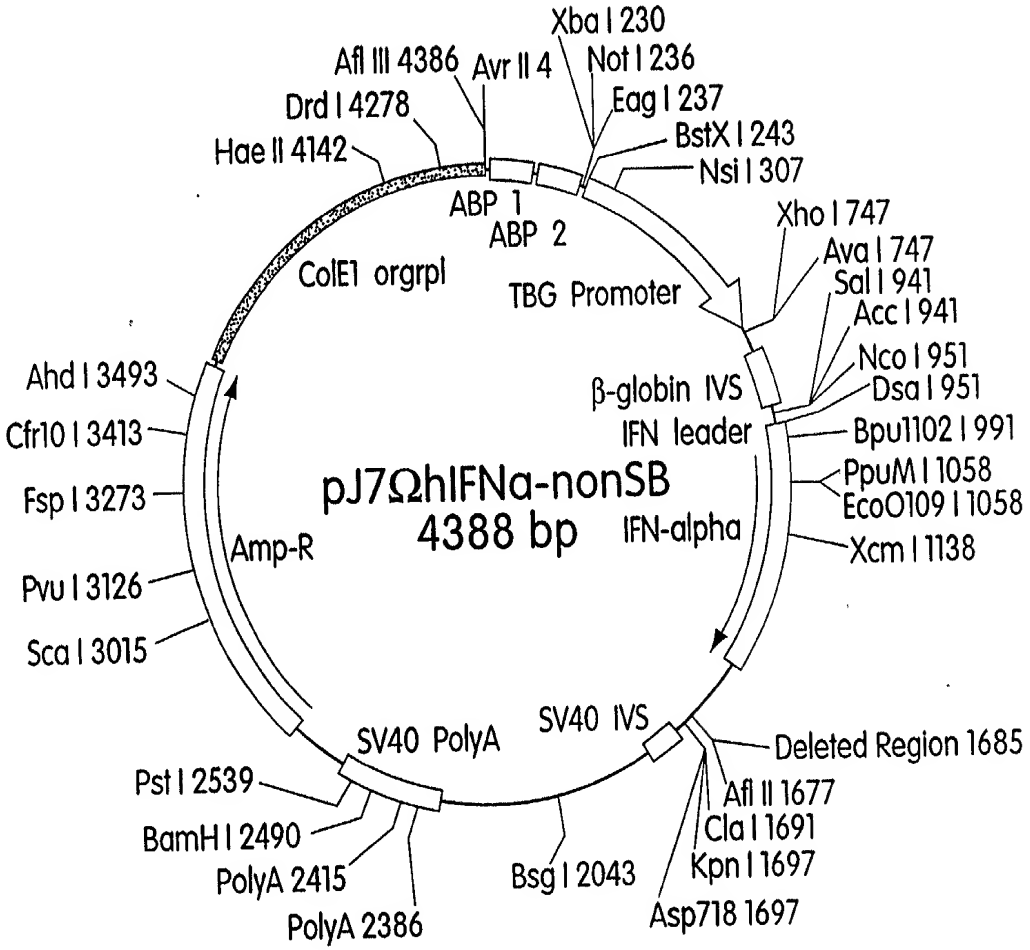
Xba I 230
Not I 236
Eag I 237
BstX I 243
Nsi I 307
Xho I 747
Ava I 747
Sal I 941
Acc I 941
Nco I 951
Dsa I 951
Bpu1102 I 991
PpuM I 1058
EcoO109 I 1058
Xcm I 1138
Deleted Region 1685
Afl II 1677
Cla I 1691
Kpn I 1697
Asp718 1697
Bsg I 2043
SV40 IVS
SV40 PolyA
PolyA 2386
PolyA 2415
BamH I 2490
Pst I 2539
Sca I 3015
Pvu I 3126
Amp-R
Fsp I 3273
Cfr10 I 3413
Ahd I 3493
ColE1 orgrpl
Hae II 4142
Drd I 4278
Afl III 4386
Avr II 4
ABP 1
ABP 2
TBG Promoter
β-globin IVS
IFN leader
IFN-alpha
pJ7ΩhIFNa-nonSB
4388 bp

Fig. 8

T092EP S2mm6660

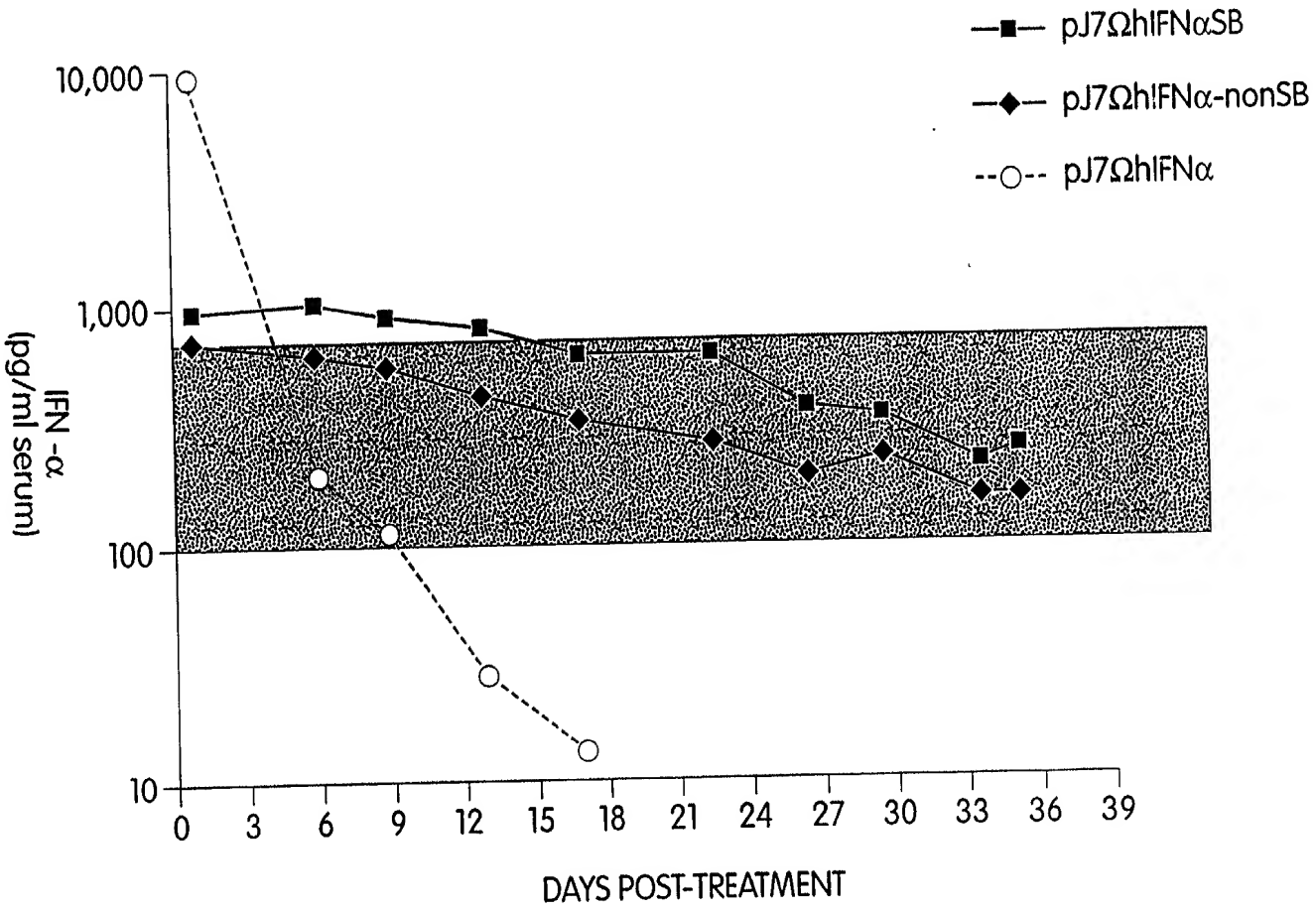

Fig. 9

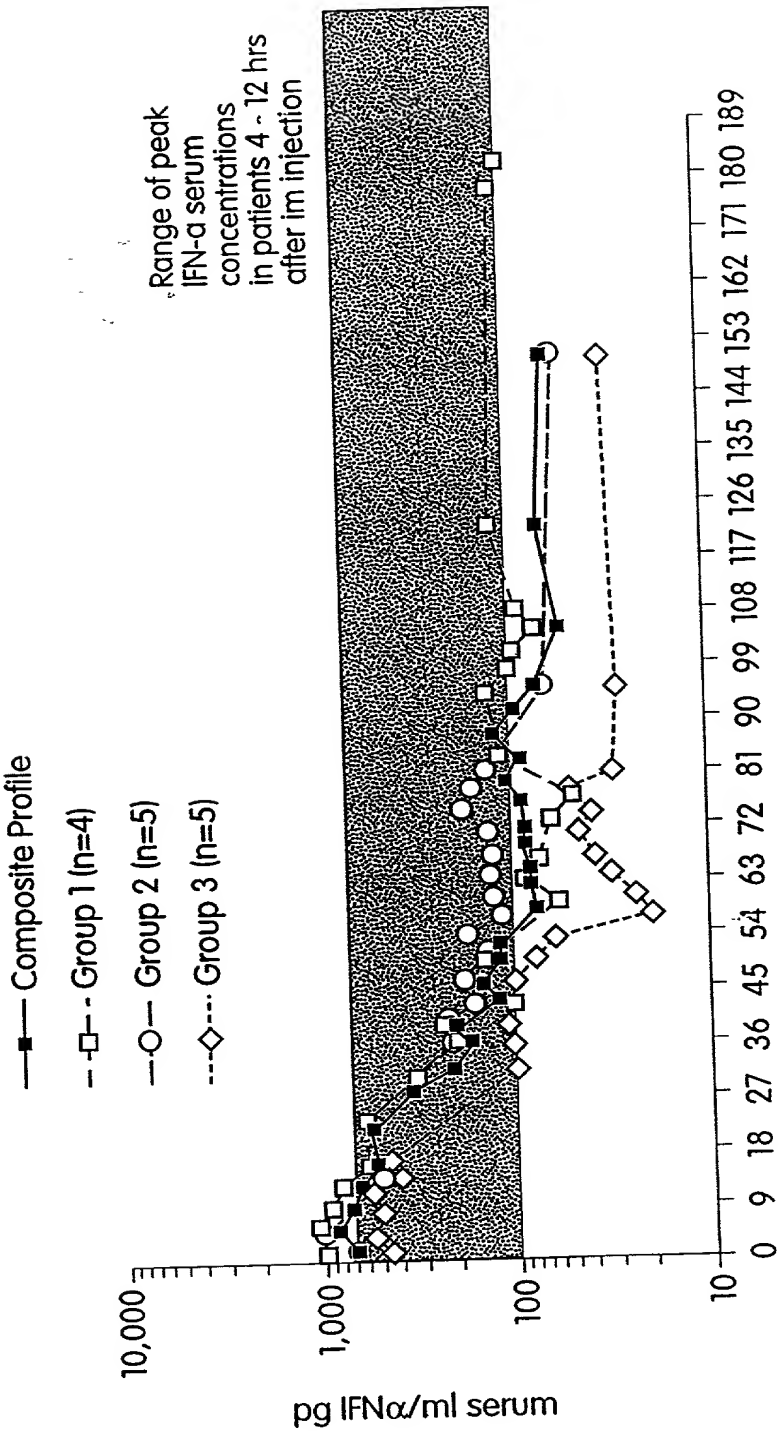
Composite Profile
Group 1 (n=4)
Group 2 (n=5)
Group 3 (n=5)
Range of peak IFN-α serum concentrations in patients 4 - 12 hrs after im injection
10,000
1,000
100
10
pg IFNα/ml serum
0 9 18 27 36 45 54 63 72 81 90 99 108 117 126 135 144 153 162 171 180 189
DAYS POST-TREATMENT

Fig. 10

T092TT "52hn6660

```
        1           11          21          31          41          51
IFN-α2  CDLPQTHSLG  SRRTLMLLAQ  MRRISLFSCL  KDRHDFGFPQ  EEF-GNQFQK  AETIPVLHEM
IFN-α1  CDLPETHSLD  NRRTLMLLAQ  MSRISPSSCL  MDRHDFGFPQ  EEFDGNQFQK  APAISVLHEL

        61          71          81          91          101         111
IFN-α2  IQQIFNLFST  KDSSAAWDET  LLDKFYTELY  QQLNDLEACV  IQGVGVTETP  LMKEDSILAV
IFN-α1  IQQIFNLFTT  KDSSAAWDED  LLDKFCTELY  QQLNDLEACV  MQEERVGETP  LMNADSILAV
          ↓   ↓
        121         131         141         151         161
IFN-α2  RKYFQRITLY  LKEKKYSPCA  WEVVRAEIMR  SFSLSTNLQE  SLRSKE
IFN-α1  KKYFRRITLY  LTEKKYSPCA  WEVVRAEIMR  SLSLSTNLQE  RLRRKE
```

Fig. 11A

		121	125	132	152	161	164	Relative Activity	Enhancement Factor
IFN-α2	α2 -------	arg	gln	lys -----	phe	ser	ser -	1	1
IFN-α2-T132	α2 -------	arg	gln	THR -----	phe	ser	ser -	0.1	0.05
IFN-α2-R125	α2 -------	arg	ARG	lys -----	phe	ser	ser	140	200
IFN<α2-K121	α2 -------	LYS	gln	lys -----	phe	ser	ser	17	15
→IFN-α2-K121R125	α2 -------	LYS	ARG	lys -----	phe	ser	ser -	400	3000
IFN-α2-R125T132	α2 -------	arg	ARG	THR -----	phe	ser	ser -	23	10
IFN-α2-K121R125T132	α2 -------	LYS	ARG	THR -----	phe	ser	ser -	170	150
IFN-α2-H125	α2 -------	arg	*HIS*	lys -----	phe	ser	ser -	9	
IFN-α2-L125	α2 -------	arg	*LEU*	lys -----	phe	ser	ser -	5	
IFN-α2-K125T132	α2 -------	arg	*LYS*	THR -----	phe	ser	ser -	4	

Fig. 11B

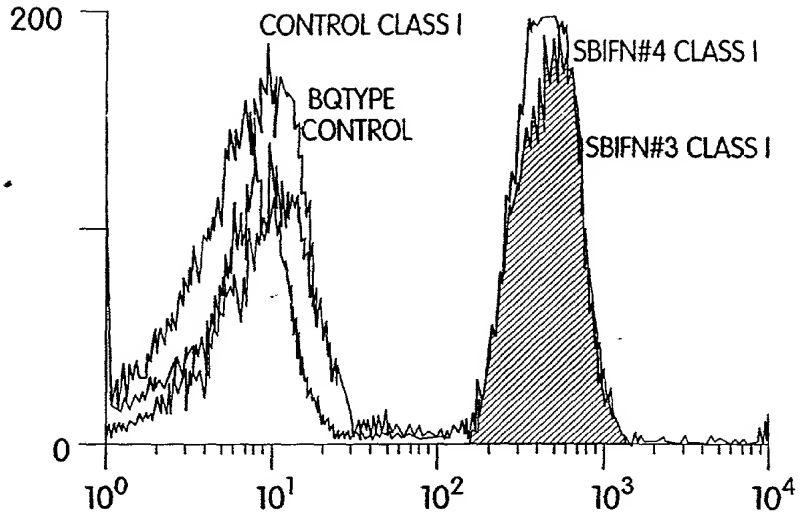

Fig. 12A

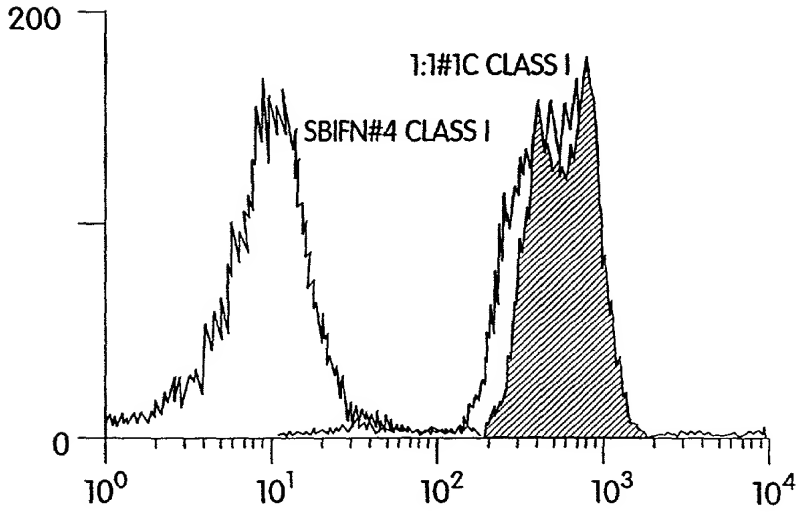

Fig. 12B

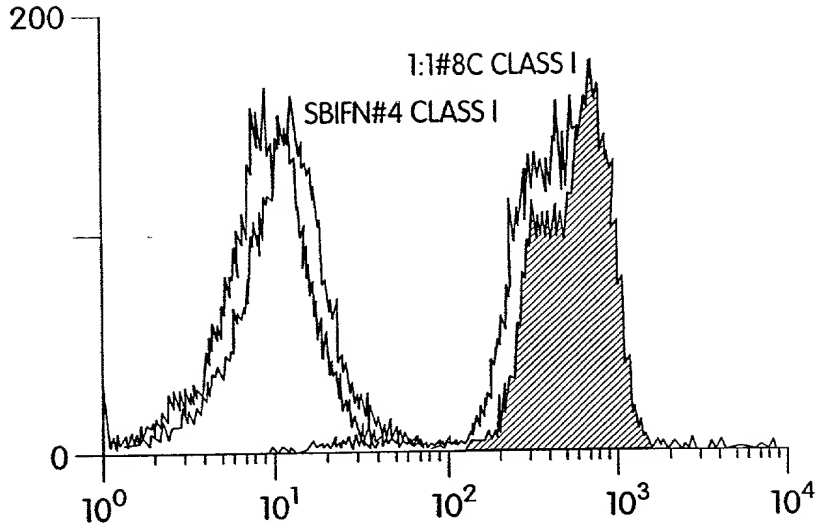

Fig. 12C

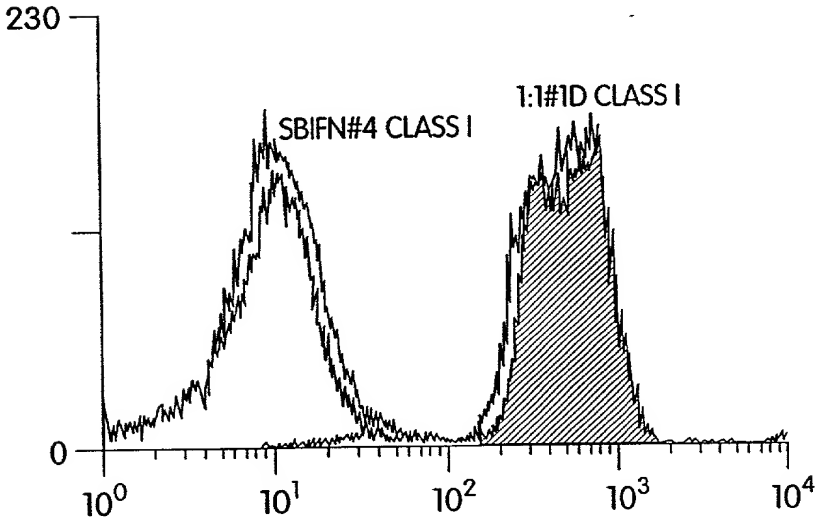

Fig. 12D

US 5,945,512

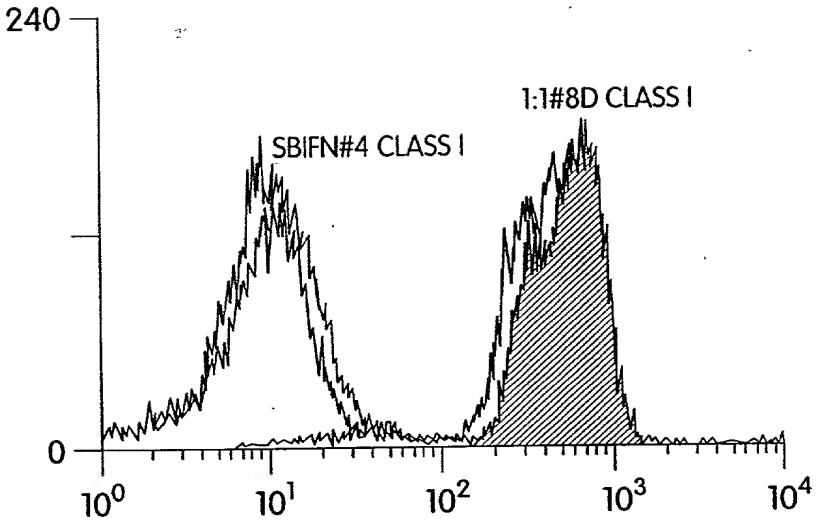

Fig. 12E

Substitute Sheet (Rule 26)